

## DIAGNOSIS

# Diagnosis of iron deficiency anaemia in hospital patients: Use of the reticulocyte haemoglobin content to differentiate iron deficiency anaemia from anaemia of chronic disease

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The diagnosis of iron deficiency anaemia in hospital patients with chronic infections and inflammation presents a challenge. Recently laboratory tests such as the reticulocyte haemoglobin content, which are independent of infection and inflammation, have become available for routine diagnostic use.

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Iron deficiency is one of the most common nutritional problems in the world and the leading cause of anaemia in children and pregnant women.<sup>[1]</sup> In South Africa (SA), iron deficiency and anaemia constitute a significant disease burden owing to chronic helminth infections and a predominantly cereal-based diet.<sup>[2]</sup>

Iron deficiency is treatable. Successful management of iron deficiency anaemia (IDA) requires accurate diagnosis followed by investigation of the underlying cause of iron loss and treatment with iron supplements. Accurate diagnosis demands differentiation of IDA from the anaemia of chronic disease (ACD), also referred to as anaemia of inflammation. In hospital patients, chronic infection(s) and inflammation often coexist with iron deficiency. In SA there is a high burden of chronic infections such as tuberculosis and HIV.<sup>[3]</sup> Anaemia has been reported in up to 95% of HIV patients, reflecting cytokine dysregulation, drug therapy, presence of infection, presence of malignancy and/or nutritional deficiencies.<sup>[4]</sup>

The distinction between concomitant iron deficiency and ACD is often difficult. Characteristically ACD is a mild to moderate anaemia (haemoglobin concentration 8.0 - 9.5 g/dL), which is normocytic and normochromic. However, ACD may also be microcytic and/or hypochromic. It is then important to distinguish it from true IDA, so that appropriate supplementation can be administered.

## Laboratory investigations

Bone marrow (BM) biopsy and iron staining is considered the gold standard test for the diagnosis of iron deficiency. However, BM biopsy is an invasive procedure and is no longer considered the standard of care for assessment of iron stores.<sup>[5]</sup> In everyday clinical practice, IDA and ACD are traditionally differentiated by assessment of iron studies, which include serum iron, transferrin, transferrin saturation and ferritin (Table 1).<sup>[6]</sup> A low ferritin level is

highly sensitive for the diagnosis of IDA, but ferritin is also an acute-phase reactant showing an increase in the presence of infection or inflammation when iron is sequestered in reticuloendothelial system macrophages. A normal ferritin level therefore does not exclude accompanying IDA.<sup>[7]</sup> This is evident by the reduced ferritin sensitivity of 46.4% at the clinically recommended cut-off of 30 µg/L in hospital patients.<sup>[3]</sup>

More recently, automated analysers can perform tests that are reported to be independent of infection and inflammation. These include biochemical parameters, namely zinc protoporphyrin and soluble transferrin receptor, as well as haematological parameters, namely the percentage of hypochromic red blood cells, and reticulocyte parameters such as the reticulocyte haemoglobin content (CHr). The advantage of measuring the haemoglobin of the reticulocyte is that the reticulocyte has a shorter lifespan (1 - 2 days) than the red cell. CHr therefore provides an early indicator of iron deficiency.<sup>[8]</sup>

## The reticulocyte haemoglobin content

The CHr is a measure of the product of the reticulocyte haemoglobin concentration and the mean cell volume. Several recent studies<sup>[8-11]</sup> have confirmed the diagnostic performance of the CHr, which is routinely available.

At the National Health Laboratory Service Haematology Laboratory at Charlotte Maxeke Johannesburg Academic Hospital, SA, we performed a prospective study in 74 hospital patients in order to compare the accuracy of the CHr with that of standard haematological and biochemical tests for the diagnosis of IDA using the BM iron stain as the reference.<sup>[3]</sup> In this study, a CHr of >28 pg reliably distinguished IDA from ACD with a sensitivity of 75.86% and a specificity of 84.10%. CHr is therefore a good discriminator of IDA.

**Table 1. Laboratory findings in, ACD and combined iron deficiency anaemia and anaemia of chronic disease (IDA and ACD)<sup>[3]</sup>**

Laboratory test	IDA	ACD	IDA and ACD
Haematology			
MCV	↓	↓ or normal	↓ or normal
MCH	↓	↓ or normal	↓ or normal
Bone marrow iron stores	↓	↑	↓ or normal
CHr (pg)	<28	≥28	<28
Hypochromic red blood cells (%)	>5	<5	>5
Biochemistry			
Iron	↓	↓	↓
Transferrin	↑	↓ or normal	↓
Transferrin saturation	↓	↓	↓
Ferritin	↓	↑	↓ or normal
sTfR	↑	Normal	Normal or ↑
sTfR-ferritin index	High (>2)	↓	High (>2)
Zinc protoporphyrin	↑	Normal	Normal or ↑
CRP	Normal	↑	↑
Hepcidin	↓	↑	↑

MCV = mean cell volume; MCH = mean cell haemoglobin; sTfR = soluble transferrin receptor; CRP = C-reactive protein.

However, the diagnostic CHr cut-off values vary according to the study population and diagnostic inclusion criteria, emphasising the importance of determining the CHr cut-off for each specific patient population.

In a study by Karlsson,<sup>[10]</sup> a higher CHr cut-off of 30.5 pg, corresponding to a sensitivity of 93% and a specificity of 69%, was shown to be indicative of IDA in 54 elderly patients. Studies performed in elderly patients have reported a higher mean cell volume (MCV) and mean cell haemoglobin (MCH) in the IDA and ACD groups.<sup>[10,12]</sup>

A study performed at Pelonomi Regional Hospital, SA, in 100 infants and children aged 6 months - 6 years showed that the optimal CHr cut-off for the diagnosis of iron deficiency was 29 pg. This corresponded to a sensitivity of 86% and a specificity of 50%, using a transferrin saturation of <25% as the diagnostic criterion for iron deficiency.<sup>[13]</sup>

The CHr has been compared with standard haematological and biochemical tests for the diagnosis of IDA. We found that the sensitivity of the CHr was not superior to the MCH parameter or the transferrin saturation, which is similar to the results of two other studies performed in hospital patients in which the authors also concluded that the CHr does not perform better than standard tests for IDA.<sup>[10,12]</sup> Further studies are required in order to identify an improved marker of iron deficiency in hospital patients compared with standard biochemical and haematological tests.

The CHr test has several advantages. It is a simple and cost-effective test. The current diagnostic panel for IDA, which includes a full blood count (FBC), peripheral smear review and iron studies, costs approximately ZAR610.00. A test panel based on the haematological parameters of FBC and CHr (as part of the reticulocyte count) costs approximately ZAR220.00. The CHr can be performed on 1 - 1.5 mL of blood in a single EDTA tube and in children a finger prick would produce an adequate sample, eliminating the need for additional tubes for the biochemical parameters.

The CHr test does have some obvious current limitations for routine use in that it can only be measured by ADVIA haematology analysers (Siemens Diagnostics, USA), the availability of which is laboratory specific. However, more recently reticulocyte parameters (Ret-He and Ret-Y) on the Sysmex haematology analyser (Sysmex Corporation, Japan) have shown good agreement with the CHr.<sup>[12,14]</sup> Also, since the CHr is calculated from the reticulocyte MCV, patients with haemoglobinopathy associated with microcytosis will have a falsely low CHr, while patients with megaloblastic anaemia or macrocytic indices (MCV >100 fl), including some patients on antiretroviral therapy, will have a falsely elevated CHr.<sup>[15]</sup> For the clinician, it is important to interpret the CHr in the context of the patient's other clinical and laboratory investigations including red

cell indices, vitamin B<sub>12</sub>, folate levels and findings on haemoglobin electrophoresis.

## Conclusion

The diagnostic distinction between IDA, ACD and the combined state of IDA and ACD in hospital patients with chronic infections and inflammation can be difficult with standard biochemical and haematological tests. Although the CHr test is not superior to standard tests for IDA, it is a simple and cost-effective alternative to biochemical and haematological parameters for the diagnosis of IDA in hospital patients. It is recommended that in hospital patients with anaemia a C-reactive protein (CRP), or other biochemical markers of inflammation, and a CHr be added to the initial FBC assessment. If the CRP is elevated, IDA can be diagnosed in patients with coexistent ACD in the presence of a CHr <28 pg and hypochromic red cell indices.

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